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Effect of $24,25(OH)_2D_3$ on PTH levels and bone histology in dogs with chronic uremia

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Effect of $24,25(OH)_2D_3$ on PTH levels and bone histology in dogs with chronic uremia. Controversy exists as to whether $24,25(OH)_2D_3$ has a direct inhibitory effect on parathyroid hormone (PTH) secretion. Therefore, the present investigation examined the effect of long-term administration of $24,25(OH)_2D_3$ on immunoassayable PTH levels (iPTH) and bone histology in dogs with chronic renal failure. Chronic renal failure was produced in 16 dogs, half of which served as controls whereas the other half received 2.5 μg/day of $24,25(OH)_2D_3$, orally. Serum iPTH, serum total, ionized calcium, serum phosphorus, and creatinine were followed at weekly or biweekly intervals in both groups. Also, creatinine clearances, serum levels of $25(OH)D_3$, $24,25(OH)_2D_3$, and $1,25(OD)_2D_3$ and the intestinal absorption of calcium were measured. After 1 year of chronic renal failure the dogs were sacrificed and rib biopsy specimens were obtained for histological examination and measurement of mineral content. Serum iPTH increased equally in the two dog groups with no effect at any time of $24,25(OH)_2D_3$ treatment, despite a significant increase in the serum levels of $24,25(OH)_2D_3$ and a concomitant decrease of the $1,25(OH)_2D_3$ levels. There was no difference in the levels of serum calcium or in the calcium content of bone. Furthermore, after 8 months of uremia three control dogs were switched to the group treated with $24,25(OH)_2D_3$ and followed for another 7 months. No suppressive effect of administering $24,25(OH)_2D_3$ on the iPTH levels could be demonstrated in these three dogs. On the rib biopsy specimens the number of osteoclasts was increased in the $24,25(OH)_2D_3$-treated dogs, compared to control uremic dogs and normal control dogs, indicating no inhibition by $24,25(OH)_2D_3$ of the effect of PTH on bone. It is concluded that long-term treatment with $24,25(OH)_2D_3$ alone in dogs with chronic renal failure has no effect either on the secretion or on the skeletal effects of PTH.

Effet de la $24,25(OH)_2D_3$ sur les niveaux de PTH et l'histologie osseuse chez des chiens en urémie chronique. Il existe une controverse quant à un effet inhibiteur direct de la $24,25(OH)_2D_3$ sur la sécrétion d'hormone parathyroïdienne (PTH). C'est pourquoi cette étude a examiné l'effet de l'administration à long terme de $24,25(OH)_2D_3$ sur les niveaux de PTH dosables immunologiquement (iPTH) et sur l'histologie osseuse de chiens avec insuffisance rénale chronique. L'insuffisance rénale chronique a été provoquée chez 16 chiens, dont la moitié servait de contrôles, tandis que l'autre moitié a reçu 2,5 μg par jour de $24,25(OH)_2D_3$ orallement. L'iPTH sérique, la calcémie totale et ionisée, la phosphorémie et la créatininémie ont été suivies à intervalles hebdomadaires ou bihebdomadaires dans les deux groupes. En plus, les clearances de la créatinine, lex niveaux sériques de $25(OH)D_3$, de $24,25(OH)D_3$, de $24,25(OH)_2D_3$, et de $1,25(OH)_2D_3$ et l'absorption intestinale du calcium ont été mesurées. Après 1 an d'insuffisance rénale chronique, les chiens ont été sacrifiés et des spécimens biopsies costales ont été obtenues pour examen histologique et mesure du contenu minéral. L'iPTH sérique a augmenté également dans les deux groupes de chiens sans effet à aucun moment du traitement par la $24,25(OH)_2D_3$, malgré une augmentation significative des niveaux sériques de $24,25(OH)_2D_3$ et une baisse concomitante des niveaux de $1,25(OH)_2D_3$. Il n'y avait pas de différence entre les niveaux de calcémie ou le contenu calci que de l'os. En outre, trois chiens contrôles ont été transférés au bout de 8 mois d'urémie, au groupe traité avec la $24,25(OH)_2D_3$ et suivis encore 7 mois. Aucun effet suppresseur de l'administration de $24,25(OH)_2D_3$ sur les niveaux d'iPTH n'a pu être démontré chez ces trois chiens. Sur les spécimens biopsies costales, le nombre d'ostéoclastes était accru chez les chiens traîtés avec la $24,25(OH)_2D_3$, par rapport aux chiens contrôles urémiques et aux chiens contrôles normaux, ce qui indique l'absence d'inhibition par la $24,25(OH)_2D_3$ de l'effet de la PTH sur l'os. On conclut que le traitement à long terme par la $24,25(OH)_2D_3$ seule chez des chiens en insuffisance rénale chronique n'a d'effet ni sur la sécrétion ni sur les effets squelettiques de PTH.

A potential interrelationship between parathyroid glands and vitamin D metabolites may play a key role in the homeostasis of calcium in humans. Since parathyroid hormone (PTH) controls the production of $1,25(OH)_2D_3$ via activation of the 1-hydroxylase, $1,25(OH)_2D_3$ or other vitamin D metabolites might in turn act directly on the parathyroid glands to inhibit PTH secretion. Divergent results have been published regarding a direct effect of $1,25(OH)_2D_3$ on PTH secretion. It has been reported that $1,25(OH)_2D_3$ inhibits [1, 2], stimulates [3], or has no effect on PTH secretion [4].

The $24,25(OH)_2D_3$ metabolite is less active than $1,25(OH)_2D_3$ [5, 6]. Recently, it has been postulated that $24,25(OH)_2D_3$ directly inhibits the secretion of PTH. Care et al [7] have demonstrated a fall in PTH secretion in goats infused with $24,25(OH)_2D_3$. Also, Canterbury et al [3] have shown that the infusion of $24,25(OH)_2D_3$ into the thyroid artery of normal dogs resulted in a rapid suppression of PTH release. Subsequently, the same investigators [8] examined the effect of administering 2 μg/day of $24,25(OH)_2D_3$ to dogs with chronic renal failure. The effect was a 40 to 60% decrease in immunoreactive PTH levels. However, other investigators have failed to demonstrate a suppressive effect of $24,25(OH)_2D_3$ on the release of PTH. Studies by Tanaka et al [9] in vitamin D-deficient and vitamin D-supplemented rats indicated that serum parathyroid hormone (i-PTH) levels did not change in response to $24,25(OH)_2D_3$.

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Henry, Taylor, and Norman [10] demonstrated that $24,25(OH)_2D_3$ alone had no effect on parathyroid glands of chicken and similar negative results were observed using cultures of rat parathyroid gland [11]. Dietel et al [12] found no effect of $24,25(OH)_2D_3$ on normal porcine parathyroid glands and human parathyroid adenomas maintained in a culture system. Our laboratory studies [13] with isolated dispersed bovine and canine parathyroid cells failed to show any effect of $24,25(OH)_2D_3$ on the secretion of PTH.

In view of the divergent results published in the literature we have carefully examined the effect of $24,25(OH)_2D_3$ on the release of PTH in uremic dogs and on the bone disease which accompanies long-term uremia.

Methods

Renal insufficiency was produced in twenty adult mongrel female dogs (weighing 17.2 ± 2.3 kg [SD]) by ligation of most of the branches of the left renal artery followed by contralateral nephrectomy 1 week later. Before the dogs were included in the investigation, it was confirmed that all had normal kidney function, normal iPTH levels, normal serum phosphate, ionized calcium, and total calcium. Three to four weeks after chronic renal failure (CRF) was introduced, creatinine clearances were measured and the 20 dogs were divided into two groups, each having a similar degree of reduced kidney function. Within the first 3 months after uremia was induced in the dogs, four died—two in each group. The remaining 16 uremic dogs survived for at least another 12 months. One month after the operation treatment with $24,25(OH)_2D_3$, 2.5 μg/day administered orally, was begun in one group of eight dogs. The other eight dogs served as a control group. After 8 months of chronic renal failure three of the dogs from the uremic control group were treated with $24,25(OH)_2D_3$ and remained on this treatment for another 7 months. All uremic dogs received normal dog chow containing 1.6% of calcium and 1.1% of phosphorus. All dogs had free access to water.

The following parameters were measured on each dog every week for 12 to 15 months: total serum calcium, ionized calcium, phosphorus, creatinine, sodium, potassium, and magnesium.

- Every second week serum iPTH (C-terminal assay) was measured.
- Every 3 months creatinine clearance was measured.
- The serum levels of $25(OH)D_3$, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ were measured after 6 and 12 months of chronic renal failure.
- Intestinal calcium absorption was measured after 6 months of uremia.
- The dogs were sacrificed after 12 to 15 months of chronic uremia and the calcium, phosphorus, and magnesium content of bone specimens from the ribs were measured. Furthermore, at the time of sacrifice rib biopsy specimens were obtained for histological examination.

Serum iPTH was measured by a COOH-terminal assay using the antibody CH9 [14]. Serum ionized calcium was measured by the Orion SS-20 electrode [15]. Total serum calcium and magnesium were measured by atomic absorption photometry. Serum creatinine, phosphorus, sodium, and potassium were measured by an autoanalyzer. Creatinine clearances were estimated by an exogenous creatinine infusion. Intestinal calcium absorption was measured by a simplified method previously described [16].

25-Hydroxy vitamin D_3 [$25(OH)D_3$] was measured by a competitive protein-binding assay [17], as were 24,25-dihydroxyvitamin D_3 [$24,25(OH)_2D_3$] [18, 19] and 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] [20].

The bone content of calcium, phosphorus, and magnesium was determined after incineration of a completely dried rib biopsy specimen, which was totally cleaned of fat and muscle. The fat was extracted by a mixture of ethanol/chloroform (3:1), and the bone was dried by air pressure and weighed (wet weight). The sample was then dried in an oven at 100°C for 24 hr and weighed again (dry weight); afterwards it was placed in a crucible oven at 600 to 700°C for 48 hr. Thereafter, 5 ml of hydrochloric acid were added to the samples, and the ion concentrations was determined by atomic absorption photometry. The results were expressed in milligrams per gram of dry weight of bone.

Bone histology was examined after approximately 1 year of chronic uremia just before the two experimental groups were sacrificed. Bone biopsy specimens were obtained from the 10th rib of each dog. Non-decalcified histological sections were prepared (5 to 10 μ thick) on a sledge microtome (Jung Model K, Heidelberg, Germany) and histometrically quantitated as previously described [21]. The following parameters were measured: *(1)* percent of relative osteoid volume, or the percent of bone matrix which was nonmineralized; *(2)* percent of total osteoid surface, or the percent of trabecular surface covered by non-mineralized matrix (osteoid); *(3)* osteoclast index, or the number of osteoclasts/mm^2 total bone area; *(4)* calcification front formation, or the percent of osteoid seam-mineralized bone interface with fluorescent tetracycline label; and *(5)* the cellular rate of mineralization or the mean distance between double fluorescent tetracycline labels divided by the interdose duration. The tetracycline labelling was performed by administering 1 g of tetracycline (500 mg every 12 hr) orally to the dogs on days 1 and 2 and again on days 14 and 15. The biopsy specimen was then obtained on day 18.

Results

The creatinine clearances in the two dog groups were equally reduced after 3 weeks of uremia and remained stable after 1 year, with a mean value of 19 ± 3.8 ml/min in the uremic control group and 16 ± 3.1 ml/min in the group treated with $24,25(OH)_2D_3$. There was no significant difference in the levels of alkaline phosphatase between the two groups. The intestinal absorption of calcium (measured after 6 months of chronic uremia) and expressed in fractional terms was 27% in the $24,25(OH)_2D_3$-treated group and 22% in the control group. However, these values were not significantly different from each other. The fractional absorption of calcium in these animals was not significantly different from the range of fractional absorption observed in six normal dogs (18 to 38%). A normal intestinal calcium absorption despite low plasma levels of $1,25(OH)_2D_3$ may, however, indicate a biologic effect of $24,25(OH)_2D_3$ in these $24,25(OH)_2D_3$-treated dogs.

The serum levels of the different vitamin D metabolites after 1 year of uremia are depicted in Figure 1. The levels of $25(OH)D_3$ were not significantly different between the two groups and were also not significantly different from the level

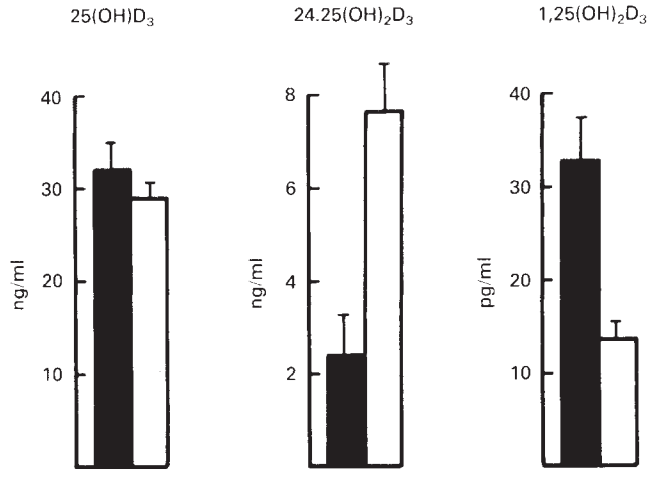

Fig. 1. *Levels of different vitamin D metabolites in plasma after 1 year of uremia in control dogs (■) and dogs treated (□) with 24,25(OH)$_2$D$_3$.* Values for 25(OH)D$_3$ were not significantly different from each other. The levels of 24,25(OH)$_2$ were significantly higher ($P < 0.005$) in the dogs receiving this metabolite. The levels of 1,25(OH)$_2$D were significantly lower ($P < 0.02$) in the treated group.

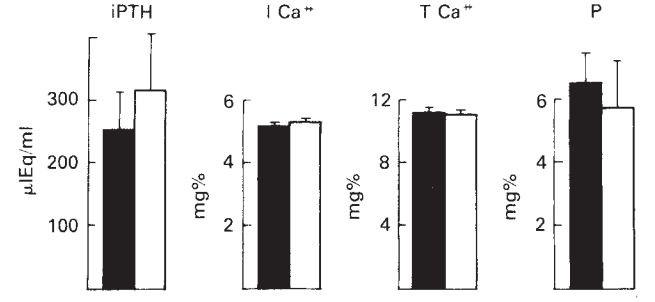

Fig. 2. *Mean serum levels of iPTH, ionized calcium, total calcium and phosphorus in uremic control dogs (■) and uremic dogs treated (□) with 24,25(OH)$_2$D$_3$ for 1 year.* No significant differences were found between the groups in any of these measurements.

observed in dogs with normal kidney function [32.8 ± 1.4 ng/ml (mean ± SE)].

The mean concentration of 24,25(OH)$_2$D$_3$ in serum was significantly higher ($P < 0.005$) in the dog group treated with 24,25(OH)$_2$D$_3$ (7.6 ± 1.1 and 2.4 ± 0.8 ng/ml, respectively) than in the uremic control dogs, but neither group was significantly different from the levels of six control dogs with normal kidney function (4.9 ± 1.3 ng/ml). In contrast to the 24,25(OH)$_2$D$_3$ levels, the serum concentrations of 1,25(OH)$_2$D$_3$ were significantly suppressed in the group of 24,25(OH)$_2$D$_3$-treated dogs ($P < 0.02$) compared to the serum concentrations in the uremic control group (13.3 ± 2.7 against 32.9 ± 5.5 pg/ml), but not significantly different compared to the level in serum of six dogs with normal kidney function (31.6 ± 13.7 pg/ml). However, this lack of a significantly difference was probably due to the large variation in the normal levels.

Figure 2 depicts the mean concentrations of serum iPTH, serum ionized calcium, total calcium, and phosphorus in the two uremic groups after 1 year of chronic renal failure. Compared to the levels in dogs with normal kidney function (<60 µlEq/ml), the iPTH levels were significantly elevated ($P <$

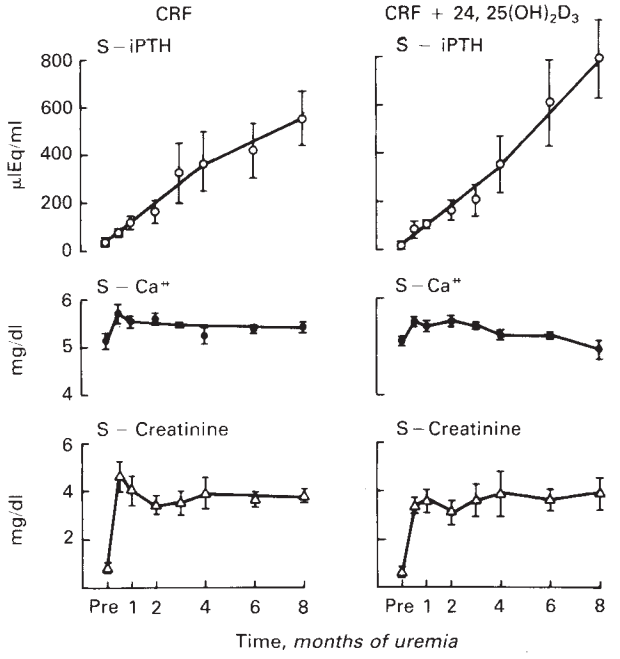

Fig. 3. *Serum levels of iPTH, ionized calcium, and creatinine in control uremic dogs (CRF) and in uremic dogs receiving 24,25(OH)$_2$D$_3$ [CRF + 24,25(OH)$_2$D$_3$] for 8 months.*

0.001) in both uremic dog groups. However, there was no significant difference between the two groups of uremic dogs, whether or not they had been treated with 24,25(OH)$_2$D$_3$. Neither the serum calcium nor phosphorus concentrations were significantly different between the two uremic groups and only the serum phosphorus differed ($P < 0.05$) from the one in normal dogs (4.7 ± 0.5 mg/dl).

Figure 3 illustrates the change in the levels of serum iPTH in the two uremic dog groups during the first 8 months of chronic renal failure. Also shown are the changes in the serum levels of ionized calcium and creatinine. The rise in serum creatinine occurred within the first month of uremia, thereafter the serum creatinine levels remained stable and were not significantly different between the two groups. The serum ionized calcium showed a small increase in both groups shortly after the reduction in renal mass and then remained stable and was not different between the two groups. Thus, as depicted in Figure 3 no effect on long-term treatment with 24,25(OH)$_2$D$_3$ could be demonstrated on the serum levels of iPTH in dogs with stable kidney function and serum ionized calcium. In both uremic dog groups the serum levels of iPTH increased from normal values to values between 600 and 800 µlEq/ml, without significant differences between the two groups.

Three of the control (untreated) dogs with chronic renal failure were administered 24,25(OH)$_2$D$_3$ after 8 months of uremia and remained on this treatment for the following 7 months. After the initial 8 months of uremia, the serum creatinine was 2 mg/dl in one dog and remained stable during the next 7 months of treatment with 24,25(OH)$_2$D$_3$. The serum creatinine concentrations were 4.1 and 4.2 mg/dl in the other two dogs at the end of the control period (8 months) and doubled during the next 7 months of treatment with 24,25(OH)$_2$D$_3$. Total and ionized serum calcium levels were

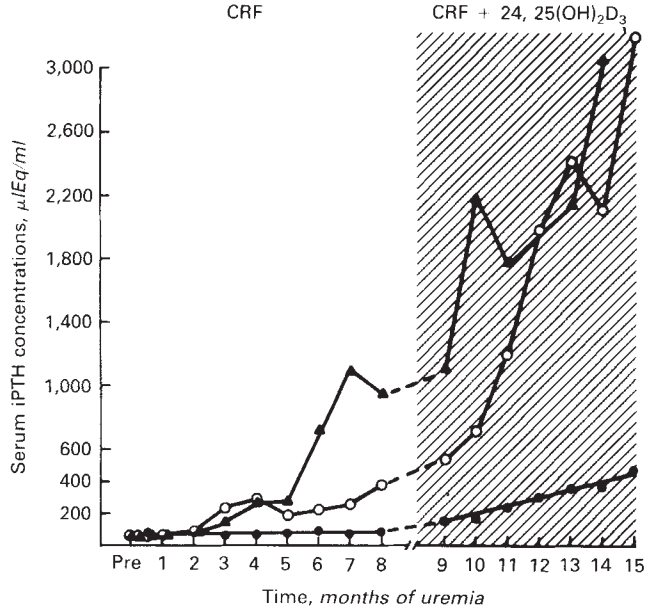

Fig. 4. *Serum iPTH levels in three dogs with chronic renal failure, before (CRF) and during treatment with 24,25(OH)$_2$D$_3$ [CRF + 24,25(OH)$_2$D$_3$].*

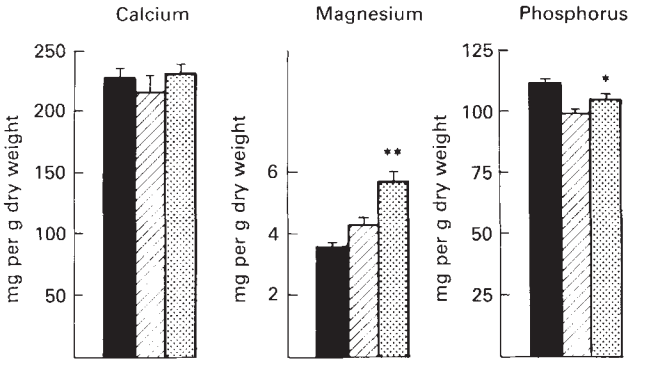

Fig. 5. *Calcium, magnesium, and phosphorus content of the rib.* The mineral content of bone from normal dogs is compared with that of dogs with chronic renal failure (CRF) and uremic dogs treated with 24,25(OH)$_2$D$_3$. There was no difference in calcium content, while the magnesium content of ribs from uremic dogs treated with 24,25(OH)$_2$D$_3$ was significantly higher than that of uremic control dogs ($P < 0.001$). Statistics between the two CRF groups are represented in the figure by *, $P < 0.05$ and **, $P < 0.001$.

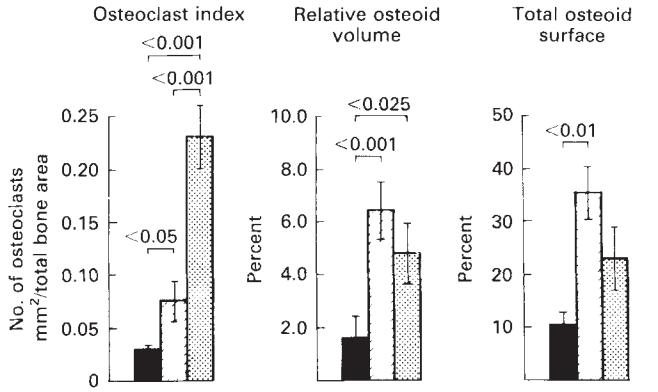

Fig. 6. *Bone histology of the ribs of normal dogs compared with the histology of ribs from dogs with chronic renal failure and of ribs from dogs with chronic renal failure treated with 24,25(OH)$_2$D$_3$.*

stable in the dog with a serum creatinine of 2 mg/dl, while the ionized calcium concentration fell from 5.0 to 4.1 mg/dl and 5.4 to 3.4 mg/dl in the two other dogs (total serum calcium fell from 12.2 to 10.7 and from 11.9 to 7.2 mg/dl, respectively). Despite treatment with 24,25(OH)$_2$D$_3$ iPTH levels increased significantly in all three dogs, demonstrating that treatment with 24,25(OH)$_2$D$_3$ did not inhibit the development of secondary hyperparathyroidism in these three dogs with either progressive chronic renal failure (two) or mild stable chronic renal failure (one) (see Fig. 4).

As shown on Figure 5 treatment with 24,25(OH)$_2$D$_3$ did not change the calcium content of bone when compared to that of normal or uremic control dogs. However, the magnesium content of bone was significantly increased ($P < 0.001$), in comparison to both uremic and normal control dogs. Similarly, the bone phosphorus content was slightly but significantly higher ($P < 0.05$) in 24,25(OH)$_2$D$_3$-treated dogs than in uremic control dogs, although both groups had a lower bone phosphorus content than normal control dogs.

The main results of the bone histology from the two uremic groups of dogs are depicted in Figure 6. The osteoclast index (the number of osteoclasts per mm^2 per total bone area) was significantly ($P < 0.005$) increased in the uremic control group, compared with normal control dogs. However, the most striking finding was a further significant increase ($P < 0.001$) in the osteoclast index of the uremic group treated with 24,25(OH)$_2$D$_3$. Thus, there was no histological evidence that 24,25(OH)$_2$D$_3$ ameliorated the skeletal consequences of the secondary hyperparathyroidism of chronic renal failure. The relative osteoid volume and the total osteoid surface (both in percent) were both significantly increased in the two uremic groups compared to normal control dogs, but not significantly influenced by treatment with 24,25(OH)$_2$D$_3$. The cellular rate of mineralization, which is a measure of the bone synthesizing capacity of the average osteoblast, was normal in both control uremic and 24,25(OH)$_2$D$_3$-treated dogs (Fig. 7). Similarly, calcification front formation which reflects the proportion of osteoid seam taking part in the mineralization process was normal in both uremic dog groups (Fig. 8).

Discussion

The present investigation clearly demonstrates that long-term treatment of uremic dogs with 24,25(OH)$_2$D$_3$ has no suppressive effect on the serum levels of PTH, as detected by a COOH-terminal PTH assay. Adequate intestinal absorption of 24,25(OH)$_2$D$_3$ was confirmed by the finding of significantly higher levels of serum 24,25(OH)$_2$D$_3$ in the treated group as compared to the uremic control group. This demonstration of a lack of a suppressive effect of 24,25(OH)$_2$D$_3$ on the PTH levels in serum agrees with the results of Tanaka et al [9] in the rat and with those of Llach et al [22] in the uremic human. Similarly, Henry, Taylor, and Norman [10] did not detect any effect of 24,25(OH)$_2$D$_3$ on the secretion of PTH or formation of cyclic AMP from normal porcine parathyroid glands or human parathyroid adenomas in organ culture. Finally, Kanis et al [23] did not notice any change in PTH levels when giving

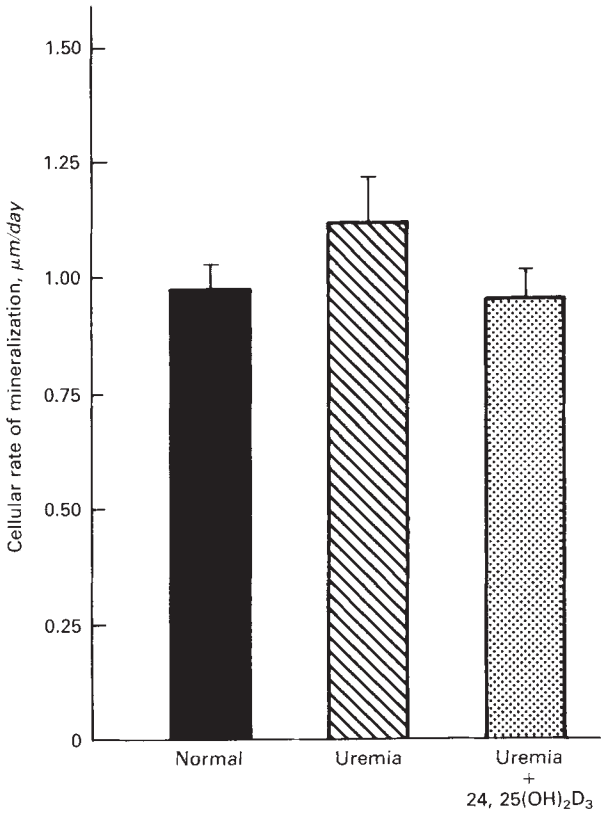

Fig. 7. *Cellular rate of mineralization as measured by the double tetracycline labeling.* The rate was not different from that of normal dogs in either of the uremic groups.

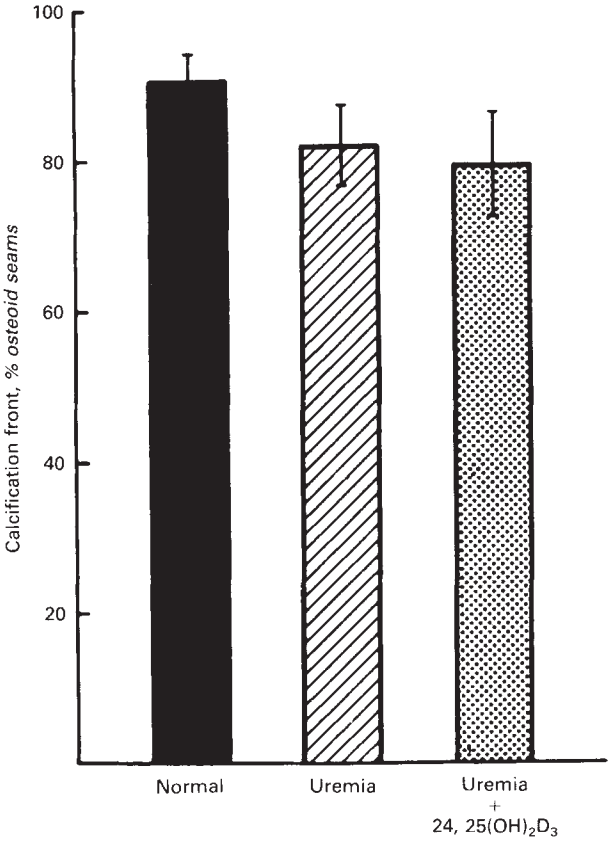

Fig. 8. *The calcification front, as measured by the tetracycline labeling.* It was not different from that of normal dogs in either of the uremic groups.

24,25(OH)$_2$D$_3$ to patients with renal disease and secondary hyperparathyroidism or to normal subjects.

However, other investigators have demonstrated a significant inhibition of PTH secretion in the dog, which was independent of changes in the serum ionized calcium after 24,25(OH)$_2$D$_3$ administration. This occurred both within minutes after an intravenous injection of 24,25(OH)$_2$D$_3$ [3] and after oral administration of 24,25(OH)$_2$D$_3$ to uremic dogs [8]. The reason for the discrepancy between the results of the last-mentioned investigation [8] and those of the present study remains to be clarified. Both investigations were performed in dogs, the degree of uremia was about the same, and the doses of 24,25(OH)$_2$D$_3$ were not significantly different. The amount of calcium in the diet was also similar in both studies. The uremic dogs of Canterbury et al [8] were treated with 24,25(OH)$_2$D$_3$ for 3 weeks and an inhibitory effect on the PTH levels was already seen after 1 week of treatment. In the present study the dogs were treated with 24,25(OH)$_2$D$_3$ for approximately 1 year and at no time during the course of the study could we demonstrate inhibition of PTH secretion.

In rats with acute uremia Pavlovitch et al [24] found that short-term administration of 24,25(OH)$_2$D$_3$ before bilateral nephrectomy prevented the rise in serum iPTH, seen in a control uremic group. Miravet et al [25] found a small, but not significant, suppression of the serum iPTH levels in patients with vitamin D-deficient osteomalacia. However, a more pronounced suppression of the serum iPTH was demonstrated during treatment with other vitamin D metabolites: 25(OH)D$_3$, 1,25(OH)$_2$D$_3$, 25,26(OH)$_2$D$_3$. Cloix et al [26] found that 24,25(OH)$_2$D$_3$ inhibited the isoproterenol- and sodium fluoride-stimulated adenylate cyclase activities in plasma membranes obtained from parathyroid adenomas of patients with primary hyperparathyroidism and from hyperplastic glands obtained from patients with chronic renal failure. Finally, Christiansen et al [27] suggested that a direct suppressive effect of 24,25(OH)$_2$D$_3$ on the parathyroid gland may exist in patients with chronic renal failure. Thus, discrepancies exist regarding the effect of short-term treatment with 24,25(OH)$_2$D$_3$ either in vivo or in vitro on PTH secretion. However, the present investigation has clearly demonstrated that during long-term treatment (1 year) there was no inhibitory effect of 24,25(OH)$_2$D$_3$ on the secretion of PTH. Furthermore, in the present investigation no effect was found on the levels of serum ionized calcium, phosphorus, and magnesium, or the calcium or phosphorus content of bones of uremic dogs. A small but significant increase in the magnesium content of the bone was found in the 24,25(OH)$_2$D$_3$-treated group. The significance of this finding remains to be clarified.

Our histological observations also indicate that 24,25(OH)$_2$D$_3$ does not ameliorate the skeletal consequences of hyperparathyroidism. For example, the cellular rate of mineralization and calcification front formation are unaffected by such treatment. We have previously shown that the only uremic patients with

normal calcification front formation are those with the highest circulating iPTH levels [28]. Furthermore, our $24,25(OH)_2D_3$-treated dogs have even more osteoclasts than do our control uremic dogs. The genesis of the $24,25(OH)_2D_3$-induced osteoclastogenesis is enigmatic but has been noted previously [29]. The lack of histological evidence of increased bone formation as determined by the percent of osteoid surface and tetracycline-based parameters indicates that the osteoclast proliferation which occurred with $24,25(OH)_2D_3$ treatment is not a consequence of the remodelling process. Specifically, remodelling is characterized by parallel changes in bone formation and resorption, an event which did not occur in our $24,25(OH)_2D_3$-treated animals. $24,25(OH)_2D_3$ is capable of directly stimulating resorption in bone organ culture at concentrations which approximate those achieved pharmacologically in our dogs [30]. Furthermore, vitamin D is known to act in concert with PTH to promote osteoclastic activity [31], and the possibility remains that $24,25(OH)_2D_3$ may exert such a synergistic effect, particularly in a milieu of severe hyperparathyroidism.

No influence on any other histological parameter could be demonstrated after treatment with $24,25(OH)_2D_3$, which agrees with the results of Muirhead et al [32] and Evans et al [33]. However, high doses of $24,25(OH)_2D_3$ did ameliorate the bone disease of vitamin D-deficient rats in the study by Fuchs et al [34] and may have a beneficial effect on the uremic bone disease connected with high aluminum loads [35, 36].

In a recent investigation on the skeletal resistance to PTH in chronic uremia, using the model of the isolated perfused canine tibia, we found no effect of long-term treatment with $24,25(OH)_2D_3$ on the diminished cyclic AMP response to stimulation by PTH 1-34 [37]. This agrees with the results of Lieberherr et al [38] in the rat calvaria, but in contrast to the results of Marcus, Orner, and Brickman [39] in rat bone.

Thus, in dogs with chronic renal failure no effect of long-term treatment with $24,25(OH)_2D_3$ could be demonstrated on: *(1)* the secretion of PTH (as measured by a COOH-terminal assay); *(2)* the PTH-induced skeletal changes of chronic uremia (as measured by an increased number of osteoclasts); or *(3)* the skeletal resistance to PTH 1-34 (as demonstrated by a reduced cyclic AMP response to stimulation by PTH) [37].

Therefore, we conclude that long-term treatment with $24,25(OH)_2D_3$, alone, in dogs with chronic renal failure has no effect either on the secretion or on the skeletal effects of PTH.

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